The evolution of reproductive structures in seed plants: a re-examination based on insights from developmental genetics

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Summary

The study of developmental genetics is providing insights into how plant morphology can and does evolve, and into the fundamental nature of specific organs. This new understanding has the potential to revise significantly the way we think about seed plant evolution, especially in regard to reproductive structures. Here, we have sought to take a step in bridging the divide between genetic data and critical fields such as paleobotany and systematics. We discuss the evidence for several evolutionarily important interpretations, including the possibility that ovules represent meristematic axes with their own type of lateral determinate organs (integuments) and a model that considers carpels as analogs of complex leaves. In addition, we highlight the aspects of reproductive development that are likely to be highly labile and homoplastic, factors that have major implications for understanding seed plant relationships. While these hypotheses may suggest that some long-standing interpretations are misleading, they also open up whole new avenues for comparative study and suggest concrete best practices for evolutionary analyses of development.

I. Introduction

The defining feature of the seed plants is the ovule, which upon fertilization develops into the seed. Yet the steps underlying the evolution of the ovule and its associated structures remain poorly understood, both in gymnosperms and angiosperms. This hampers our ability to relate reproductive structures across clades of seed plants, and thus, to reconstruct the evolutionary history of this key group. Here, we argue that insights from developmental genetics are essential to resolving long-standing questions in plant systematics and paleobotany, and that conversely, a broad understanding of systematics and paleobotany can guide comparative developmental studies into productive avenues.

Several major questions have driven research in seed plant systematics and paleobotany for over a hundred years, including: How does the angiosperm carpel relate to ovule-bearing structures in gymnosperms? Is the fundamental nature of the flower a branched or simple axis? How did hermaphroditism evolve and in how many lineages? Along these lines, the genetic bases of phenomena such as determinacy and branching have been the subjects of developmental evolutionary studies, but this work has largely focused on recently diversified angiosperms (Yoon & Baum, 2004; Vollbrecht *et al.*, 2005; Sliwinski *et al.*, 2006; Kellogg, 2007; Sliwinski *et al.*, 2007), primarily on close relatives of established genetic models. These studies take advantage of research using model systems that has begun to unravel the inherent logic of plant development, and illuminate the processes by which plants have diversified. While some researchers have begun to integrate molecular findings regarding developmental processes into studies of leaf and root evolution (Rothwell *et al.*, 2008; Sanders *et al.*, 2009; Boyce, 2010), we

believe that the time is right to expand this effort to aspects of reproductive evolution. In particular, functional genetic data have the potential to inform the way we assess the distinction between homology vs. homoplasy - the common inheritance of features as opposed to their independent evolution. As a starting place, we summarize emerging developmental genetic insights into how angiosperm reproductive structures are formed, modified, and recombined. Next, we consider how these findings impact our thinking about the evolution of ovules, ovule bearing structures, and various aspects of flowers. Finally, we discuss how these insights bear on our understanding of reproductive structures in seed plants and on the design of developmental evolutionary studies.

II. Transformation and transference in angiosperm developmental genetics

The complementary phenomena of homeosis and modularity are the fundamental mechanisms by which plants build their bodies (Walbot, 1996; Baum & Donoghue, 2002). Seed plants reiteratively produce a basic module, the phytomer, which is composed of three subunits: the lateral determinate organ, the axillary meristem and the associated internodal stem (Gray, 1879). Plants then generate morphological complexity via the differential expression of genetic identity programs that alter developmental patterns within the subunits. This mechanism is inherently homeotic; it depends on a sequential transformation of identity (Sattler, 1988). For instance a meristem may initially produce juvenile leaves, then mature leaves, then bracts, then floral organs. All of these structures are lateral determinate organs but their identity, and hence their morphology, differ based on which genetic program is expressed, both in the organs and

in the meristem that produces them. Although any one species may only express relatively few alternate identity programs (e.g., *Arabidopsis thaliana*, Fig. 1), across the seed plants there are dozens, if not hundreds, of potential variations (e.g., a vegetative meristem can be a thorn, tendril, branch; Gifford & Foster, 1988; Bell, 1991). The expression of these genetic programs is controlled by a diverse array of endogenous (e.g., determined by age, position) and exogenous (e.g., determined by light quality, photoperiod, temperature) pathways. Transitions between identities may be abrupt, as with the conversion of an inflorescence meristem to floral meristem identity (Kaufmann *et al.*, 2011), or gradual, as with the effect of phase change on leaf morphology (Huijser & Schmid, 2011). Another important point is that the high degree of modularity we observe at the morphological level is also reflected at the genetic level. As modules themselves, genetic programs display a high degree of spatial and temporal lability, and can even be transferred across subunit boundaries, such as with the expression of meristematic activity in a lateral organ (see below). In the following brief overview, we highlight the most critical aspects of the genetic programs controlling reproductive identity and development, in *Arabidopsis thaliana* with an emphasis on their homeotic and modular natures (see Table 1 for a summary of the major genes or gene families discussed herein). This background provides a framework for our subsequent discussion of evolutionary models.

1. The genetic basis of the phytomer

From a genetic perspective, the best understood subunits of the phytomer are the meristem (whether primary or axillary) and the lateral organs. While the expression of

identity programs such as “inflorescence” or “bract” may vary in time and space, the genetic pathways that control meristematic activity appear to be common to all meristems and, likewise, the fundamental patterning of lateral organ primordia is the same across diverse organ types (these two subjects have been reviewed in detail by Barton, 2010; Kidner, 2010; Moon & Hake, 2011, from which the following discussion is drawn unless otherwise noted). Typical angiosperm shoot apical meristems can be subdivided into the so-called central zone (CZ), in which cells divide slowly and maintain a pluripotent state, and the peripheral zone (PZ), which is marked by more rapid divisions of undifferentiated cells and is the site of lateral primordium initiation (Fig. 2A). The CZ genetic module is composed of a non-cell autonomous receptor pathway, which involves several receptor kinases and their peptide ligand CLAVATA3 (CLV3), and the homeodomain transcription factor WUSCHEL (WUS). While *WUS* acts to promote CZ identity, the *CLV3* pathway acts to restrict it. These opposing actions are accomplished via a homeostatic feedback whereby WUS function activates *CLV3* expression, which in turn acts to represses *WUS*, resulting in a maintained balance of CZ activity. In contrast, the undifferentiated state of the PZ is promoted by a subfamily of homeodomain loci called the type I KNOX genes. Specifically, the main players are members of two paralogous gene lineages respectively defined by the *Arabidopsis* gene *SHOOTMERISTEMLESS* (*STM*) and the maize gene *KNOTTED1* (*KN1*), which are expressed throughout the meristem except in incipient primordia. These two key genetic pathways, the *WUS/CLV* module acting in the CZ and the *STM/KN1* genes maintaining the PZ, work together to establish the activity and integrity of all shoot apical meristems (Fig. 2A).

The initiation of lateral organs requires down-regulation of the meristematic program, namely via repression of KNOX gene expression in the PZ. In fact, the localized elimination of S*TM/KN1* expression is one of the earliest markers for a shift in PZ cell fate towards leaf identity. Several genetic pathways involving complex signaling responses underlie KNOX down-regulation. One of these is based on polar auxin transport (PAT), a phenomenon whose broader significance for plant development cannot be overstated. The polarized, cell-to-cell trafficking of auxin, mediated by the PINFORMED (PIN), P-glycoprotein ABC transporter (PGP) and AUX protein families, allows the phytohormone auxin to be concentrated in specific cells. Depending on the identity of these cells, a peak in auxin concentration can induce a wide range of developmental responses (reviewed in Grunewald & Friml, 2010). In the PZ of the meristem, auxin flows primarily through the outer epidermal layer, oriented towards the PZ. An auxin concentration peak in this region induces the formation of a new primordium (Fig. 2A). As the organ begins to develop, the inductive auxin flows away through the central core of the primordium, which both defines the new vasculature of the leaf and drains auxin away from the immediate region of the PZ. This local auxin depletion creates the so-called primordium "inhibition zone" and results in the stereotypical phyllotaxy of any given meristem by preventing the establishment of new auxin peaks in the immediate vicinity of a recently initiated leaf (Kuhlemeier, 2007). The auxin peak associated with a new primordium also initiates the down-regulation of KNOX gene expression. In addition, the activation of the primordium developmental program up-regulates the expression of a suite of genes that feedback negatively onto the KNOX loci to reinforce their repression while simultaneously acting to establish the

adaxial (upper) and abaxial (lower) surfaces of the incipient leaf. The juxtaposition of opposing abaxial and adaxial identity is essential for the lateral expansion that produces the lamina. The major genetic players on the adaxial surface include the Arabidopsis genes *ASYMMETRIC LEAVES1* (*AS1*), a MYB transcription factor, and its co-factor *ASYMMETRIC LEAVES2* (*AS2*), a LOB domain transcription factor, along with the class III homeodomain leucine zipper-containing (HD-ZIPIII) genes. Working in opposition on the abaxial side are members of the so-called KANADI and YABBY transcription factor families (Fig. 2A). Lastly, there are genes that act at the junction between the growing primordium and the meristem, most notably members of the *CUC SHAPED COTYLEDON* (*CUC*) gene family, which repress cell divisions and thereby promote separation of the primordium from the meristem.

The fundamental meristem and primordium genetic programs do not function alone, but work in concert with additional identity programs that determine what *kind* of meristem or leaf will be produced. Such identity programs may impinge directly on the genes mentioned above or they may work in parallel. For instance, vegetative meristem identity will impact a meristem's response to auxin flow in order to produce spiral phyllotaxy, while a switch to floral meristem identity may alter this response to yield whorled phyllotaxy. Beyond these interactions are some perhaps surprising modifications that can blur the very definition of meristem and leaf. Early work on compound leaf development demonstrated that it is associated with reactivation of the PZ KNOX genes in the developing leaf primordium (reviewed Champagne & Sinha, 2004; Koenig & Sinha, 2010). Extensive studies have now shown that this involves the wholesale transference of the genetic module controlling PZ identity and primordium

initiation into the leaf itself, thereby creating a limited degree of indeterminacy and allowing discrete leaflet formation (reviewed in Rosin & Kramer, 2009). Moreover, this translocation has occurred in numerous independent instances, although there are a handful of compound leaves that rely in part on additional genetic mechanisms (e.g., Champagne *et al.*, 2007). Another fascinating aspect of the PZ/primordium genetic module is that it also apparently underlies a wide spectrum of what can be called complex leaves, ranging from dissected to lobed to toothed, to even the bizarre morphologies of the Podostemaceae and *Streptocarpus* (Harrison *et al.*, 2005; Katayama *et al.*, 2010). These observations underscore how the modularity of plant developmental genetic programs can enable extreme levels of morphological lability by simply shifting their localization.

2. Determinate vs. indeterminate growth and inflorescences

One of the most fundamental meristematic alterations occurs in the transition from vegetative to reproductive meristem identity. This can happen in two ways: the meristem can be directly converted from vegetative to floral identity or it can transition first to inflorescence meristem identity before floral meristems are formed. While both vegetative and inflorescence meristem identity programs can be considered indeterminate, once a meristem has acquired floral meristem identity, it is, by definition, determinate and primary growth terminates. Further distinctions are often made between determinate and indeterminate inflorescences but in the former case, the inflorescence typically becomes determinate by transforming itself into floral identity. In *Arabidopsis thaliana*, the inflorescence is considered indeterminate. Its developmental program

differs dramatically from that of the vegetative meristem in that internodes are elongated, lateral organs are largely suppressed, and lateral meristems are immediately active rather than suppressed as in the rosette. The first few nodes of the inflorescence produce a lateral leaf subtending a secondary inflorescence meristem but that pattern quickly transitions to the production of lateral floral meristems with no subtending leaves. These floral meristems produce yet another completely different pattern – strongly condensed internodes, lateral organs with floral organ identity and suppressed axillary meristems (Fig. 1).

The genetic basis for the switch from inflorescence meristem identity to floral meristem identity is the differential expression of complementary identity programs. These genetic modules are complex but, in Arabidopsis, inflorescence identity is defined by expression of loci such as *TERMINAL FLOWER1* (*TFL1*), *AGL24* and *SUPPRESSOR OF CONSTANS* (*SOC1*) (Lee & Lee, 2010), while floral meristem identity is primarily promoted by *LEAFY* (*LFY*) and *APETALA1* (*AP1*) (reviewed in Moyroud *et al.*, 2009; Moyroud *et al.*, 2010). Both genetic studies and modeling demonstrate that differential expression of these two identity programs can account for the full range of inflorescence structure diversity in angiosperms (Prusinkiewicz *et al.*, 2007; McKim & Hay, 2010). For example, the cymose *Aquilegia formosa* inflorescence meristem produces two bracts, each with an axillary inflorescence meristem, then converts to floral meristem identity, allowing the axillary inflorescence meristems to repeat the pattern (Ballerini & Kramer, 2011). In groups with especially complex inflorescence structure, such as the grasses, there appear to be more than one genetic "flavor" of meristem identity (e.g., primary branch, secondary branch, spikelet, floret, etc.), but the model is similar – differential

expression of these various identities has allowed the generation of enormous morphological diversity (Vollbrecht *et al.*, 2005; McSteen, 2006; Hake, 2008). Interestingly, double mutant combinations of *LFY* or mutations in other loci that help promote floral meristem identity can result in meristems that possess some floral organs along with unusual patterns of branching. For example, in flowers of *ap1* mutants, the outer whorls contain bracts and axillary meristems while the inner whorls produce normal stamens and carpels (Irish & Sussex, 1990). It is important to appreciate that these phenotypes are not atavistic but, rather, the product of mixed genetic identity, a reasonably common outcome that results when floral or inflorescence identity genes are misexpressed. These phenotypes do underscore, however, how fine is the line between determinate and indeterminate meristem development and how easily this line can be blurred via complete or partial genetic transformation.

3. Floral organ identity

The ABC model of floral organ identity is one of the best-understood genetic programs in plants (reviewed in Causier *et al.*, 2010; Litt & Kramer, 2010; Liu & Mara, 2010), from which the following discussion is drawn unless otherwise noted). This model holds that three main classes of gene activity are expressed in floral meristems in overlapping domains such that they create a combinatorial code corresponding to each floral organ type. Sepals are determined by A function; petals, by A+B; stamens, by B+C; and carpels, by C alone (Fig. 2B). In addition to these canonical ABC classes, we now recognize that another gene class, termed the E class, is broadly expressed in the floral meristem and acts to facilitate the ABC functions. The majority of these genes are

members of the type II subfamily of MADS box containing transcription factors: *APETALA1* (*AP1*) in the A class; *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) in the B class; *AGAMOUS* in the C class; and the *SEPALLATA1-4* (*SEP*) loci in the E class. Although aspects of the model have been significantly revised, especially the nature and conservation of A function (Davies *et al.*, 2006; Litt & Kramer, 2010), it is critical to appreciate that floral organ identities are understood to be entirely interchangeable, with simple shifts in gene expression allowing complete homeotic transformations. It is important to note that organ position and number are largely controlled independently of the ABC model, i.e., floral organ identity is overlaid on primordia whose number and position are controlled by separate genetic pathways.

4. Elaboration of the carpels

The carpel is a highly derived structure that is distinctive among seed plants. It comprises a chamber enclosing the ovules (the ovary), a transmitting tract through which the pollen tubes grow, and a stigmatic surface, which receives and mediates recognition of the pollen. While the identity of the carpel is established by C+E function, many aspects of the genetic pathways controlling carpel development are based directly on the systems controlling lateral organ development (reviewed in Ferrandiz *et al.*, 2010, from which the following discussion is drawn unless otherwise noted). The general principles of these programs are common across all phylloid organs, but in several cases carpel-specific paralogs have evolved to control lateral organ development. For instance, the Arabidopsis YABBY family member *CRABS CLAW* (*CRC*) has become largely carpel-specific. In addition to the canonical YABBY role in the determination of abaxial

identity, *CRC* acts immediately downstream of the C class gene *AG* to promote the identity of the carpel itself. This is not to say that *CRC* is the only YABBY gene that contributes to carpel development, but it is the only family member whose role is largely restricted to carpel development. The existence of carpel-specific paralogs may serve to reduce genetic pleiotropy, allowing the carpel developmental program to evolve in dissociation from other lateral organs. Other types of organ polarity pathways contribute to the development of the stigma and transmitting tract (reviewed in Ferrandiz *et al.*, 2010), but little comparative work has been done on them to date, even within angiosperms (but see Fourquin *et al.*, 2005).

One especially fascinating aspect of carpel differentiation is the specification of the placenta, the tissue that will give rise to the ovules. *Arabidopsis* placental tissue is derived from a region positioned in a crease that forms between the carpel wall and replum/septum of the silique (Fig. 2C). This is part of a broader domain with apparent meristematic activity that is termed the medial ridge. From a genetic perspective, this region has all the hallmarks of axillary meristem – it arises in association with the adaxial surface of a lateral organ (the inner surface of the carpel wall) and it expresses many of the genetic markers associated with the peripheral zone of shoot apical meristems, including type I KNOX genes and patterns of differential auxin trafficking. Expression of components of this PZ vs. primordium regulatory system in the carpel appears to be associated with the complex elaboration of the carpel, specifically the maintenance of indeterminacy that is required for placenta development and subsequent ovule production (Skinner *et al.*, 2004; Girin *et al.*, 2009). In many ways, this makes the carpel analogous to complex leaves where the PZ/primordium genetic program is expressed in a lateral

organ in order to maintain a degree of indeterminacy. Although both the complex leaf and carpel developmental programs use the PZ/primordium module, they differ fundamentally in terms of their products: in leaves, the meristematic zone produces leaflets or lobes but no new lateral meristems, whereas in carpels, this zone produces new lateral meristems – the ovules (see below) – but no leaflets. This distinct difference may be conditioned by the expression of the PZ module within the context of vegetative identity on the one hand and female reproductive identity on the other, much as with behavioral changes of apical meristems between vegetative and floral identity. The apparent co-option of the PZ program in carpels again highlights the modularity of meristematic identity and the diversity of developmental functions for which it can be deployed.

5. The fundamental nature of the ovule

The ovule is an indehiscent, integumented megasporanium in which a nucellus surrounds one or few functional megaspores; integuments initiate from the chalaza to form a micropyle through which pollen tubes transport either motile or nonmotile sperm. The identity of ovules in *Arabidopsis thaliana* is established in large part by the collective function of one or more *AG*-like loci, which in Arabidopsis include *AG* itself as well as the related paralogs *SHATTERPROOF1/2* (*SHP1/2*) and *SEEDSTICK* (*STK*) (Pinyopich *et al.*, 2003). Because the *AG/SHP* and *STK* lineages diverged before the diversification of the flowering plants (Kramer *et al.*, 2004), most angiosperms have one or more representatives of *AG* as well as at least one ortholog of *STK*. What is particularly interesting is that most *STK* homologs studied to date have ovule-specific expression

patterns and, in some taxa, these genes appear to function alone in defining ovule identity (reviewed in Kramer *et al.*, 2004). However, in *Arabidopsis*, this function is redundantly encoded by *AG*, *SHP1/2* and *STK*, suggesting that ovule identity, sometimes termed "D" function (Colombo *et al.*, 1995), is not necessarily a distinct role of the *STK* lineage. At the same time, the existence of multiple loci that can contribute to ovule identity may, again, act to reduce pleiotropy and allow ovule development to evolve in dissociation from that of the carpel.

As already noted, an important point of divergence in the carpel-as-complex leaf analogy is that ovules are not modified leaflets. Rather, they are their own kind of meristematic axis, as indicated by several features (Fig. 2D). For one, ovules express *WUS*, marking the nucellus as an analog of the CZ (Gross-Hardt *et al.*, 2002). For another, they produce their own lateral organs, the integuments, the formation of which is dependent on *WUS* just like the formation of leaf primordia in apical meristems (Gross-Hardt *et al.*, 2002). Interestingly, this form of indeterminacy does not appear to involve the KNOX genes and is therefore distinct from what we see in complex leaves and carpels. In *Arabidopsis*, *WUS* is both necessary and sufficient for integument initiation: over-expression of the gene within the ovule results in the production of an indeterminate number of additional integuments (Gross-Hardt *et al.*, 2002; Sieber *et al.*, 2004). There are important differences, however, between *WUS* function in a typical meristem and its role in ovules. Most importantly, there is no evidence for a role of the *CLV3* feedback pathway in ovules (Gross-Hardt *et al.*, 2002), which may reflect the fact that unlike indeterminate shoot meristems, the ovule is distinctly determinate. After the adjacent chalazal domain produces a small number of lateral organs, the nucellus is entirely

consumed by the production of the megaspore and subsequent megagametophyte, or is modified to store reserves for the latter.

As expected for lateral organs produced in conjunction with a meristematic axis (in this case the ovule), the integuments express significant components of the genetic program that patterns lateral organs. Both the inner and outer integuments depend on the establishment of abaxial and adaxial identity for their proper development, although the exact complement of participating loci differs between the two organs (Fig. 2D; Kelley *et al.*, 2009). In the *Arabidopsis* inner integument, multiple HDZIPIII loci act in the adaxial domain while the KANADI gene *ABERRANT TESTA SHAPE* (*ATS*) determines abaxial identity. The outer integument appears to depend on the HDZIPIII *REVOLUTA* (*REV*) for adaxial identity and the YABBY gene *INNER NO OUTER* (*INO*) along with several KANADI homologs for abaxial identity, which is responsible for producing the proper curvature of these anatropous ovules. Similar to *CRC* in the carpel, *INO* is a YABBY gene that has become functionally restricted to integument polarity (Villanneva *et al.*, 1999). In addition to these fundamental markers of lateral organ identity, both integuments depend on expression of the AP2/EREBP transcription factor *AINTEGUMENTA* (*ANT*), which is also expressed in all lateral organs (Elliott *et al.*, 1996). Thus, we see that the ovule exhibits developmental and genetic parallels to a modified meristematic axis that produces a limited number of lateral organs.

III. Implications for understanding patterns of seed plant evolution

What do these genetic insights into reproductive development imply for seed plant evolution, specifically the nature of integuments, ovules, and associated structures? Ovules across seed plants likely are homologous, given that analyses of morphological data from both living and extinct taxa have supported their monophyly (e.g., Crane, 1985; Nixon *et al.*, 1994; Rothwell & Serbet, 1994; Doyle, 1998; Hilton & Bateman, 2006; Doyle, 2008). However, it remains unclear how integuments, which are variable in number across seed plants, are related to one another and, similarly, what is the correspondence among ovule-bearing structures of different clades. At higher levels of organization, questions remain as to how transitions from unisexual to bisexual as well as branched to unbranched axes were achieved.

1. Integuments

Integuments enclose the nucellus and form the micropylar tube through which pollen travels toward the egg cell. Presumed ovule precursors of the earliest seed plants lacked integuments that fully enclosed the nucellus and have, therefore, been called pre-ovules (Stewart & Rothwell, 1993). The nucellus of pre-ovules was subtended by fused or partially fused appendages, which have been viewed by many as being derived by condensation and reduction of a group of branches or dichotomously branching telomes (e.g., Andrews, 1963; Smith, 1964; Rothwell & Scheckler, 1988; Stewart & Rothwell, 1993), or a group of megasporangia (Kenrick & Crane, 1997). Under this view, the integuments were thought to have originated by subsequent fusion of these appendages.

The genetic evidence that ovules have characteristics of meristems (Gross-Hardt *et al.*, 2002) suggests an alternative hypothesis regarding the nature and origin of

integuments. Specifically, integuments, as well as the sterile appendage of pre-ovules, could be lateral organs initiated by nucellar meristems, and are of de novo origin. The nucellar meristem appears to result from co-option of portions of the CZ genetic module into the megasporangium developmental program. Further, given that the overexpression of *WUS* results in additional integuments (Gross-Hardt *et al.*, 2002; Sieber *et al.*, 2004), the dynamics of *WUS* expression in the ovule could explain both the origin of the inner integument and the variable number of integuments observed across seed plants. This variation includes a wide diversity in integument number ranging from the second integument of angiosperms to the supernumerary integuments of taxa nested within otherwise unitegmic clades, such as Taxaceae and gnetophytes (Coulter & Chamberlain, 1917b; Takaso, 1985; Takaso & Bouman, 1986; Yang & Jack, 2004) as well as the extinct Bennettitales and Erdtmanithecales (Friis *et al.*, 2011), to third integuments in ancestrally bitegmic clades such as Annonacaeae (Endress, 2011). The question of whether *WUS* homolog expression in the nucellus is conserved across angiosperms and in other clades of seed plants is critical to testing this concept of ovules and their integuments (Table 2). Notably, the *WUS*-like gene from *Gnetum* is expressed in the apex of the developing ovule primordium, indicating that this role may in fact be conserved; limited data from other gymnosperms suggests they possess *WUS*-like genes, but their expression patterns are yet to be determined in detail (Nardmann *et al.*, 2009).

Patterns of ovule ontogeny from *Gingko*, gnetophytes, conifers, and angiosperms are completely consistent with this view of integuments and their origin. In all gymnosperms and angiosperms that have been examined, the ovule primordium clearly initiates before the integuments, which subsequently arise from the flanks of the nucellus

(Coulter & Chamberlain, 1917b; Takaso, 1985; Takaso & Bouman, 1986; Takaso & Tomlinson, 1989a; Takaso & Tomlinson, 1989b; Takaso & Tomlinson, 1990; Takaso & Tomlinson, 1991; Tomlinson, 1992; Tomlinson *et al.*, 1993; Yang, 2004; Douglas *et al.*, 2007; Rydin *et al.*, 2010; note, comparable developmental data from cycads are lacking). In this way, the initiation of the nucellus and integuments is very like the initiation of apical meristems and lateral organ primordia (Steeves & Sussex, 1989).

In Arabidopsis, expression patterns of leaf polarity genes in the integuments (Fig. 2D) also support the interpretation of integuments as lateral organs, as does the presence of *ANT* transcripts in both leaves and integuments (Elliott *et al.*, 1996). A common feature of leaves and integuments is the expression of HDZIPIII and KANADI genes in the respective adaxial and abaxial surfaces of inner and outer integuments (Fig. 2D; Kelley & Gasser, 2009; Kelley *et al.*, 2009). It is important to note, however, that unlike leaves, neither Arabidopsis integument utilizes the adaxial identity locus *AS1* and the inner integument lacks YABBY expression. In regard to the *AS1* gene, it may be that the lack of PZ identity in the ovule negates a requirement for *AS1* to down-regulate the KNOX genes. In the case of the differences between the inner and outer integument, these could reflect a fundamental difference in their derivation, perhaps with the inner being derived from branches (e.g., Kelley & Gasser, 2009), as predicted by the telomic theory of origin (see above). However, it is equally possible that the developmental programs of the outer and inner integuments have diverged due to their different morphology or simply as a result of developmental system drift (True & Haag, 2001). An added complication is that the YABBY lineage itself is seed plant-specific (Floyd & Bowman, 2007) and it is unknown how the timing of its appearance relates to the

origination of either integument. Further data from angiosperms and gymnosperms are clearly needed to distinguish among the alternatives (Table 2). The polarity genes are members of gene families with complex evolutionary histories (Floyd & Bowman, 2007; Yamada *et al.*, 2011), and while expression of the YAB locus *INO* appears to conserved across angiosperms (Yamada *et al.*, 2003), it remains to be determined if expression patterns of other polarity genes are similarly conserved in flowering plants. Likewise, few data exist about the distribution and expression of polarity genes outside of angiosperms, although *ANT* has been detected in gymnosperm integuments (Shigyo & Ito, 2004; Yamada *et al.*, 2008).

2. Ovules

Developmental geneticists often use interchangeably the phrases, "female identity" and "carpel identity", but clearly, female identity is determined by the presence and development of a megasporangium, a structure that long predates the origin of seed plants, let alone carpels. Therefore, it may be more productive to hypothesize the following. 1) Female identity in seed plants is determined by the elaboration of a meristematic tissue, the placenta, which initiates one or more ovules. 2) Expression of this basic female identity program leads to the modification of the formerly sterile surrounding tissues, and this pattern of modification has evolved along different trajectories in various clades of seed plants, leading to diverse reproductive architectures. The questions then become: What genetic pathways lead to elaboration of the placenta and initiation of the ovule, and are they shared across seed plants? As with questions about integuments, full characterization of candidate gene families in terms of evolution

and expression patterns is needed to identify common determinants of ovule identity (Table 2). Regardless of the outcome, the results will set the stage for subsequent experiments to investigate how the basic female identity program interacts with other meristem and organ identity genes to produce the architectures found in different clades of seed plants.

There is limited evidence that allows us to consider the genetic basis for ovule identity. As discussed above, this so-called "D" function is closely associated with homologs of the *AG* subfamily but it is important to remember that the often ovule-associated *STK* lineage is derived from an angiosperm-specific duplication event. Gymnosperms possess *AG* family members that predate this duplication, but these have experienced their own independent duplication events (Winther and Kramer, unpub data). Based on the Arabidopsis model (Pinyopich *et al.*, 2003), we would expect members of the *AG* lineage *s.l.* to determine ovule identity in other seed plants. Consistent with this, data available from conifers suggest that multiple *AG*-like genes are broadly expressed in both male and female cones, with expression becoming more localized to different tissues as development proceeds (Rutledge *et al.*, 1998; Jager *et al.*, 2003; Zhang *et al.*, 2004; Englund *et al.*, 2011; Groth *et al.*, 2011). This would seem to indicate that in gymnosperms, *AG*-like genes are acting in the entire reproductive axis, but more sampling and better detail in expression patterns will be important for accurate interpretation of these findings, especially in light of previously unrecognized complexity that has been detected within the conifer *AG*-like gene lineages (Winther and Kramer, unpub. data). In addition to testing *all* the *AG*-like paralogs, it would be equally important to investigate other components of the ovule identity and development pathway

(reviewed in Skinner *et al.*, 2004; Kelley *et al.*, 2009) to gain an understanding of their potential functions across seed plants.

3. Ovule-bearing structures

In living seed plants, ovules are variously borne on the inner walls of carpels (angiosperms), on leafy or reduced megasporophylls (cycads), on axillary stalks subtended by leaves (*Ginkgo*), at the termini of condensed axillary shoots (gnetophytes), or on the surface of a cone scale that represents a condensed axillary shoot (conifers). What genetic pathways might interact with those that determine female reproductive identity to shape this architecture? And exactly how do variations in the pathways and their interactions result in the variety of reproductive architecture observed in seed plants?

To address these questions, let us first return to our characterization of the carpel as a complex leaf that uses the PZ genetic module in a female reproductive context, which we could simply call "PZ+C." This is an intriguing model but considerable additional work is required in angiosperms to determine whether it is broadly applicable. Keeping that significant caveat in mind, it is still interesting to examine how the PZ+C model might help explain the diversity in ovule bearing structures. First, if we consider the PZ module alone, we know that it can be expressed in two completely different contexts: in terminal or axillary meristems it helps drive the production of entire phytomers, while in leaves, it plays a more narrow role in promoting leaflet/lobe initiation. What if the PZ+C module is similarly labile? The laminar megasporophylls of angiosperms evolved from within a diverse assemblage of seed plants that were

themselves apparently derived from lineages that produced terminally-borne pre-ovules (Friis *et al.*, 2011). What if the PZ+C module first arose in the context of a meristem rather than a lateral organ? This hypothesis would hold that when PZ+C is expressed in a meristematic context, it can produce an ovule-bearing stalk, either axillary or terminal, but when co-opted into a lateral organ, would produce a laminar structure bearing ovules, similar to what we see in angiosperm carpels or cycad megasporphylls. While this idea is, admittedly, highly speculative, it does suggest specific lines of investigation into the nature of ovule production in extant gymnosperms, as well as potential explanations for the genetic basis of diversity seen in fossil seed plants.

The first area of needed research concerns the nature of female reproductive identity. Although we typically think of "C" function as primarily related to *AG* homologs, which have already been discussed, carpel identity is also promoted by the YABBY paralog *CRC*. Orthologs of this gene are expressed in all angiosperm carpels examined to date, including those of members of the ANITA grade (Yamada *et al.*, 2004; Fourquin *et al.*, 2005; Ishikawa *et al.*, 2009; Yamada *et al.*, 2011). Functional tests are more limited but are still consistent with a model that *CRC*'s role in carpel identity is broadly conserved, although in certain lineages it may perform additional developmental functions (Yamaguchi *et al.*, 2004; Lee *et al.*, 2005; Orashakova *et al.*, 2009). Current data suggest that the *CRC* lineage is angiosperm-specific, without obvious gymnosperm precursors (Yamada *et al.*, 2011), so it is critical to obtain a more detailed picture of the YABBY lineage in gymnosperms in order understand the origin of their role in carpels.

A useful starting place for a discussion of the role of the PZ+C module in the diversification of seed plant female structures is with a description of those structures. A

megasporophyll, leafy or reduced is the fundamental ovule-bearing structure in both angiosperms and cycads. As with the carpel, the leafy megasporophylls of *Cycas* are candidate analogs of complex leaves expressing the PZ/lateral primordium pathway along the margins. In more distal positions along the megasporophyll, leaflets are produced, while in more proximal positions, ovules arise. In contrast, a modified axillary shoot is the fundamental ovule-bearing structure shared by Ginkgophytes, conifers (living and extinct), and gnetophytes. In *Ginkgo biloba* the ultimate product of modification is a stalk bearing a pair of ovules, with each stalk borne in the axil of a leaf. In conifers, ovule-bearing stalks of the axillary shoot were fused with sterile subtending scales into a cone-scale, which in turn was more or less fused with the bract that originally subtended the axillary shoot, leading to a branch-scale complex. The branch-scale complex is the basic unit of the conifer cone and they are variously aggregated to produce the diversity of cones in modern conifers. In gnetophytes, axillary shoots, with terminal ovules subtended by sterile scales, are condensed and aggregated into cones of varying degrees of laxness, i.e., more or less elongated and condensed.

The starting point for these structures is thought to have been a lax axillary shoot similar to that of extinct Cordaitales (e.g., Florin, 1951; Clement-Westerhoff, 1988), and analyses of combined morphological and molecular data suggest that *Ginkgo*, conifers, and gnetophytes share a common ancestor with Cordaitales (Mathews *et al.*, 2010), as do some analyses of morphological data alone (Doyle, 2006; Hilton & Bateman, 2006; Doyle, 2008). Inasmuch as ovules in Cordaitales were terminal (e.g., Florin, 1951; Stewart & Rothwell, 1993), these observations indicate that living gymnosperms may represent two basic trajectories in the evolution of reproductive architecture, one in which

the placental/ovule meristem pathways have been transferred onto the megasporophyll, as may have happened in cycads and angiosperms, and one in which these pathways have been maintained in an essentially terminal position. This suggests that a synthetic understanding of the evolution of reproductive development may require at least three models, one each for angiosperms and cycads, and one for a gnetophyte, conifer or *Gingko*. This should begin by characterization of the relevant gene families in gymnosperms, followed by documentation of expression patterns of their members. Intuitively, we might predict the greatest similarity between cycads (particularly *Cycas*) and angiosperms, with type I KNOX and *CUC* genes expressed along the margins of the megasporophyll. Conversely, the cones of conifers and gnetophytes and the stalked ovules of *Ginkgo* represent compound structures for which the question is whether KNOX gene expression is associated with the tissues that immediately give rise to the ovules.

4. Hermaphroditism

Hermaphroditic axes occur in angiosperms, gnetophytes, and Bennettitales, and are occasionally observed in some conifers. Nonetheless, dioecy and monoecy predominate in seed plants. The two most recent models to explain the transition from dioecy and monoecy to hermaphroditism in angiosperms are the Mostly Male (MM) and the Out of Male/Female (OOM/F) models (Frohlich & Parker, 2000; Theissen *et al.*, 2002; Theissen & Melzer, 2007). The MM was based on a premise of ectopic identity expression rather than complete homeosis, specifically that ovule identity was expressed on the surface of a microsporophyll which subsequently became sterilized to enclose the ovule. Although

key aspects of this model have been definitively disproven (Vazquez-Lobo *et al.*, 2007), it represents a critical first step in the process of integrating developmental genetic data into our understanding of angiosperm evolution. The OOM/F model makes a clear case for homeosis as the driving force underlying the evolution of hermaphroditism. Quite simply, a male strobilus could become hermaphroditic if B homolog expression was eliminated from the distal sporophylls or, alternatively, a female strobilus would become hermaphroditic if B homologs were ectopically expressed in proximal sporophylls (Theissen *et al.*, 2002). Baum and Hileman expanded on this idea to produce a more detailed model for how such a shift in gene expression might have occurred in terms of transcriptional regulation (Baum & Hileman, 2006).

How can we determine whether the OOM/F model is accurate? Ideally, we would manipulate expression of homeotic B class homologs in gymnosperms to test whether such simple transformations are possible but, unfortunately, no extant gymnosperms are currently tractable for functional genetics. In lieu of such tests, we might consider the predictions of a homeotic identity program. Most notably, we would expect the occurrence of hermaphroditic teratologies, as are observed throughout angiosperms. In fact, this has been well documented: occasional bisexual strobili are observed throughout conifers, and also in *Gnetum*, most commonly represented by male cones that have distal sporophylls transformed to female identity (reviewed in Coulter & Chamberlain, 1917a; Flores-Renteria *et al.*, 2011; Rudall *et al.*, 2011). In these cases, the proximal lateral organs have fertile microsporophyll identity while the distal nodes have fertile ovule identity. Although it is yet to be decisively demonstrated, the expectation is that these transformations are the result of differential expression of homologs of B-class homeotic

genes. Other types of teratologies have been described in *Ginkgo*, where normally unisexual short shoots produce both male and female organs, albeit on separate strobili, and in other cases, chimeric leaves bear ectopic ovules. The former case could be explained by inconsistent expression of B gene homologs within the short shoot axillary meristem while the latter could result from imprecise delimitation of leaf boundaries within the short shoot meristem (Douglas *et al.*, 2007). This would be analogous to mutants of *Arabidopsis* where perturbation of primordium positioning can result in chimeric organs (Levin & Meyerowitz, 1995; Wilkinson & Haughn, 1995), although in the case of *Ginkgo* it would be a chimera of leaf and axillary female strobilus. Homoplastic evolution of hermaphroditism also provides evidence that components of the homeotic program may be widely conserved. Perhaps the most notable examples of this are *Welwitschia* and some species of *Ephedra*, which express a cryptic bisexuality much like the moneocy of angiosperms (Endress, 1996). Furthermore, many extinct lineages exhibit forms of bisexuality, including representatives of the Bennettitales (Friis *et al.*, 2011). Thus, consistent with the lability inherent in such a homeotic identity program, bisexuality appears to be homoplastic.

IV. Understanding the origin of the flower

The bisexual flower is a canonical angiosperm structure in which the carpels are subtended by whorls of microsporangia (in stamens) and sterile bracts (petals, sepals). Is the flower derived from a branched (pseudanthial origin) or unbranched (euanthial origin) axis? We believe that this question may not be especially critical given the high degree

of flexibility inherent in the genetic program that controls development of such differences. Simple shifts in the expression of lateral organ and/or meristem identity can rapidly convert branched to unbranched axes and vice versa. This provides a simpler explanation than complicated reduction series or axial condensation to derive the angiosperm flower.

As noted by Boyce (2010): "Determinacy is the ancestral sporophyte condition, its suppression for indeterminate growth was an important early innovation, and resumption of determinacy has always been present for the differentiation of sporangia." This point has been elegantly demonstrated by genetic studies in *Physcomitrella* that targeted loci involved in epigenetic remodeling of the genome. Deletion of the *Physcomitrella* Polycomb Repressive Complex 2 member *CURLY LEAF* (*PpCLF*) results in the activation of the sporophyte developmental program in the gametophytic stage of the life cycle (Okano *et al.*, 2009). If these aberrant plants are maintained in culture, they form branched bodies with multicellular "stems" that are quite unlike what is observed in normal gametophyte branching. However, if *PpCLF* function is restored, the pseudo-sporophyte will switch back to determinate development and produce a sporangium-like structure, albeit a sterile one due to the fact that the tissue is haploid. These findings underscore the idea that indeterminate development is what happens when sporangial identity is delayed, and further suggest a global switch for these transitions – epigenetic remodeling – that is conserved across land plants (Jarillo *et al.*, 2009).

So how is this developmental switch between indeterminacy and determinacy expressed in seed plants? As discussed in section III, many extant and fossil taxa produce lateral strobili that are ultimately determinate, although the axes vary in their degree of

elongation (Friis *et al.*, 2011). In male strobili, microsporophyll identity tends to be expressed in the first order lateral organs to produce a simple axis (Fig. 3A, see 3B for an exception). By contrast, in female strobili, the expression of megasporophyll and/or megasporangium identity is often delayed by one or more orders of branching until after the production of subtending sterile organs, although simple unbranched female axes certainly do occur (Fig. 3C-F). This diversity of patterns is entirely in keeping with the homeotic nature of the phytomer. Although the sporangium developmental program, whether male or female, is inherently determinate, the expression of that program is sometimes accelerated or delayed, which generates diversity in reproductive structures.

From a genetic perspective, determinacy in angiosperm flowers is established by the floral meristem identity gene *LFY* via activation of the C function gene *AG*, which later in development initiates a pathway that represses expression of the CZ gene *WUS* (reviewed in Ferrandiz *et al.*, 2010). Furthermore, both suppression of axillary meristems in the flower and compression of internodal elongation appear to be a component of floral meristem identity, genetically established by *LFY* along with *AP1* and other loci (Moyroud *et al.*, 2009; Moyroud *et al.*, 2010). To understand the implications for other seed plant structures, we need to determine how widely these functions are distributed. Gymnosperms have two types of *LFY*-like genes, termed *LFY* and *NEEDLY* (*NLY*) (Mouradov *et al.*, 1998; Frohlich & Parker, 2000), that are broadly expressed in both male and female reproductive axes, including strobilus apical meristems and both sterile and fertile lateral structures (Mouradov *et al.*, 1998; Dornelas & Rodriguez, 2005; Vazquez-Lobo *et al.*, 2007). Thus, it is possible that *LFY* homologs commonly control degrees of branching and internodal length but, while more expression data will be

useful, ultimately functional data from gymnosperms would be required to definitively test this possibility. On the other hand, some floral meristem identity genes, most notably *AP1*, are angiosperm-specific (reviewed in Litt, 2007), raising the potential that certain components of the indeterminacy vs. determinacy switch did evolve in the common ancestor of angiosperms prior to their diversification.

As to *AG*-like genes and *WUS*, the former have been found to be broadly expressed in the reproductive axes of several conifers, and species of *Cycas*, *Gingko* and *Gnetum* (Rutledge *et al.*, 1998; Jager *et al.*, 2003; Zhang *et al.*, 2004; Englund *et al.*, 2011; Groth *et al.*, 2011), and it does appear that *WUS*-like genes are expressed in male and female structures of *Gnetum* (Nardmann *et al.*, 2009). In this context, it is interesting that another observed conifer teratology is the reversion of reproductive cones to vegetative identity, which results in indeterminacy of the axis (Rudall *et al.*, 2011). The genetic basis of these mutant forms is unknown but could rely on either *AG* or *LFY/NLY* homologs. Regardless, their existence suggests that determinacy and reproductive identity go hand in hand for gymnosperms as well as angiosperms. Obviously, our understanding of the evolution of the *AG* and *WUS* gene lineages in gymnosperms is still limited and further experiments would be useful to track the expression of *WUS*-like genes during strobilus development. Even in angiosperms, the role of *AG* in repressing *WUS* is not immediate but delayed until after carpels have initiated (Lenhard *et al.*, 2001; Lohmann *et al.*, 2001), so shifts in the timing of this repression could result in axes of variable lengths. It is completely unknown whether the mechanism by which *AG* represses *WUS* is conserved across angiosperms, let alone gymnosperms (Table 2), but it would be very interesting to see whether variation in this module underlies variation in

reproductive axis length in other taxa. For instance, such shifts might underlie the difference in the condensed cones of *Welwitschia* and *Ephedra* versus the elongated cones of *Gnetum*.

V. Conclusions

Over the last twenty years, several striking themes have emerged from phylogenetic studies. One of these is that homoplasy is ubiquitous (Wake 2009). Even complex morphological and physiological syndromes appear to have evolved independently (e.g., heteroarthrocarpy, Hall *et al.*, 2011; C4 photosynthesis, Sinha & Kellogg, 1996; double fertilization, Friedman, 1990; Carmichael & Friedman, 1996; succulence, Nyffeler *et al.*, 2008). Likewise, we have all been struck by previously unforeseen relationships between wildly disparate morphological forms (Bremer *et al.*, 2009) – Rafflesiaceae and euphorbs? *Nelumbo* and *Platanus*? The examples go on. The underpinning of both these phenomena is the developmental genetic lability of plant development, whose modular nature facilitates evolutionary exceptionalism. By fully integrating a molecular genetic viewpoint into the study of seed plant reproductive evolution, we can gain new insights and identify more productive lines of research. In the development of the ovule, we recognize its meristematic nature and the likelihood that integuments can be added de novo. This frees us from the necessity of identifying a precursor for the outer integument of angiosperms and raises the possibility that the presence of multiple integument-like structures may well be homoplastic. Consideration of the carpel suggests that it is a complex lateral organ associated with a placental meristem that utilizes a PZ-like genetic

module. Our understanding of the homeotic basis of floral organ identity demonstrates that the apparently dramatic evolution of hermaphroditism was probably accomplished via undramatic, simple shifts in gene expression, likely multiple times independently. Lastly, the simple unbranched flower does not have to be explained with complex series of condensation and intermediates. Transitions between branched and unbranched axes can be achieved, again, through simple shifts in gene expression. We can recognize that such differences in branching patterns may evolve too rapidly to be phylogenetically informative.

The homoplasy of integument number and hermaphroditism on the one hand, and the lability of ovulate structural morphology and determinacy on the other, changes the traditional images that have guided the search for the sister group of angiosperms. For instance, given the lability of integument number, this precursor need not have a cupule that could be converted to an outer integument, or be a gymnosperm with multiple integuments. Such insights should also guide how we consider character and character states for phylogenetic analyses. In seed plants, where so much of the diversity needed to understand their evolution is extinct, character evolution will be understood best in synthetic analyses that combine molecular data for their statistical power with morphological data for the diversity of taxa that can be included. Obviously, more paleobotanical research is crucial since every new discovery has the potential to change the way we think about seed plant evolution, and improving our understanding of individual extinct taxa will empower the phylogenetic analyses. Likewise, we desperately need to improve our understanding of reproductive developmental genetics in extant gymnosperms so that the insights gained thereby can inform our understanding of

character evolution. Functional tools are sadly lacking at this time, but we currently know so little that there are plenty of questions to pursue. Transcriptomic projects underway have the potential to substantially improve our understanding of gene lineage evolution and, hopefully, this can be paired with comparative gene expression studies. Ideally, we would produce a detailed atlas of gene expression patterns (e.g., of *LFY/NLY*, MADS, *WUS*) in reproductive axes across multiple gymnosperm lineages, beginning with investigation of the questions outlined in Table 2. However, just as gymnosperms resist functional analyses, they are also not the most tractable systems for in situ hybridization. This may indicate that other methods, such as laser microdissection, would be fruitful for such studies. Of course, there are also several major aspects of reproductive morphology, such as the transmitting tract and stigmatic surface, which have received little attention. Given that analogs of the stigma occur in both extinct and living gymnosperms (Takaso & Bouman, 1986; Endress, 1996; Friis *et al.*, 2011), comparative studies could provide insight into whether the stigma in angiosperms simply represents a redeployment of a more broadly conserved seed plant program for pollen reception or, likewise, whether any gymnosperm reproductive tissues share process homology with the transmitting tract. Lastly, we believe it is critical to sample as many taxa as possible in order to achieve the most robust reconstruction of ancestral seed plant expression patterns. While some answers may remain beyond our grasp, recognizing the most constructive questions will allow considerable progress towards the goal of understanding the evolutionary processes that drove the most significant radiation in land plants.

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Table 1: Arabidopsis Genes or Gene Families Discussed in the Text

Arabidopsis Locus	**Gene Family**	**Functions**
WUSCHEL (*WUS*)	WOX homeodomain	- Central zone identity in shoot apical meristems - Integument production in ovules
SHOOTMERISTEMLESS (STM)	KNOX homeodomain	- Peripheral zone identity in shoot apical meristems - Maintained indeterminacy in complex leaves - Meristematic activity of the placenta
HDZIPIII	Class III homeodomain leucine zipper	- Adaxial organ identity in lateral organs, incl. leaves, floral organs and integuments
CUP SHAPED COTYLEDON (CUC)	NAC domain	- Separation of lateral primordia incl. leaves, leaflets and ovules
CRABS CLAW (CRC)	YABBY	- Aspects of carpel identity and abaxial identity
INNER NOOUTER (INO)	YABBY	- Abaxial identity of the outer integument

ABERRANT TESTA SHAPE (ATS)	KANADI GARP-domain	- Abaxial identity of the inner integument
AINTEGUMENTA (ANT)	AP2/EREBP	- Growth and proliferation in all lateral organs, including leaves, floral organs and integuments
LEAFY (LFY)	LFY	- Floral meristem identity, incl. control of phyllotaxy, floral organ identity and determinacy
TERMINAL FLOWER1 (TFL1)	PEBP	- Inflorescence identity, indeterminacy in meristems
APETALA3 (AP3) PISTILLATA (PI)	Type II MADS box	- Petal and stamen identity
AGAMOUS (AG)	Type II MADS box	- Carpel and ovule identity, floral meristem determinacy

See text for relevant references.

Table 2: Major Outstanding Questions for Comparative Investigations of Reproductive Development

1) Are *WUS* homologs expressed in ovules across the seed plants?
2) How are YABBY genes expressed in gymnosperm integuments and megasporophylls and what do these patterns tell us about the evolution of the discrete *CRC* and *INO* functions in angiosperms?
3) How conserved are KNOX gene expression patterns in the tissue giving rise to ovules across the seed plants? What about other components of the PZ module such as *CUC* genes and auxin trafficking?
4) Are teratological bisexual gymnosperms associated with differential expression of B-class gene homologs?
5) How conserved are genetic pathways controlling determinacy vs. indeterminacy (e.g., *LFY*, *AG*, *TFL1*-like genes) across seed plants?

Figure Legends

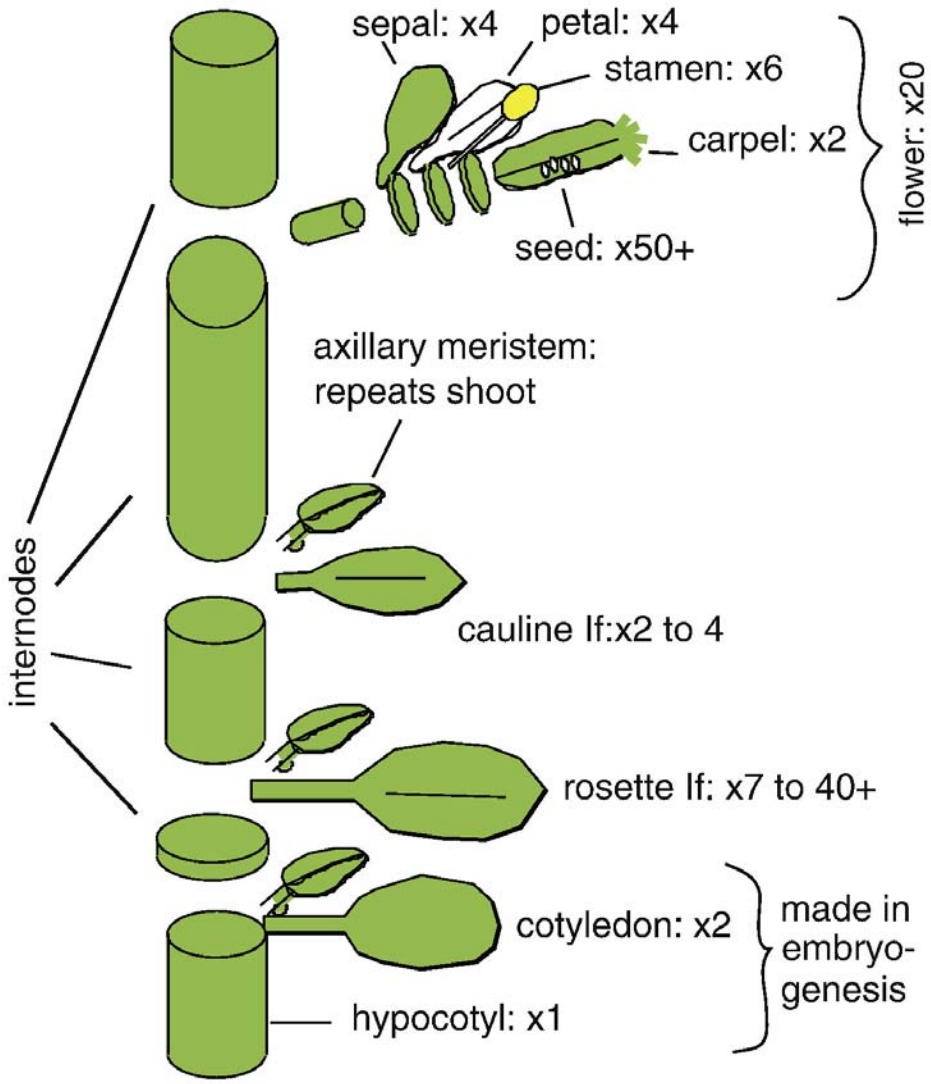
Figure 1. Parts list for the *Arabidopsis* shoot. All above ground parts of the plant, except the hypocotyl and cotyledons, are made from the shoot apical meristem. Leaves are located at nodes with stem segments, or internodes, between them. The number of rosette leaves depends on ambient environmental conditions that influence time to flowering. Axillary meristems are made in the leaf axil—the junction of leaf and stem. Reprinted from Barton 2010 by permission of the author.

Figure 2. A. Schematic of a meristem in longitudinal section. Stem cell activity is repressed by the secreted CLAVATA3 (CLV3) peptide (fushia), which acts to limit the size of the *WUSCHEL* (*WUS*) zone (pink). *WUS* promotes stem cell activity and positively regulates *CLV3* activity, thus generating a feedback loop that stabilizes stem cell activity in the meristem. KNOX gene expression (yellow) marks the PZ of the meristem and interacts with *WUS* activity via the hormone cytokinin. The positions of new leaves (P0 and P1) are marked by peaks in auxin concentration (green). These initiating primordia are delimited from the meristem by the expression of leaf/meristem boundary genes (dark purple). Within developing leaves, YABBY and KANADI genes (blue) act on one surface to establish abaxial identity while the HDZIPIII and AS1 loci (red) act on the other to determine adaxial identity. Modified from Barton, 2010 and reprinted with permission of the author. B. The ABC model as it relates to Arabidopsis floral structure (reviewed in (Krizek & Fletcher, 2005). A+E determine sepal (se)

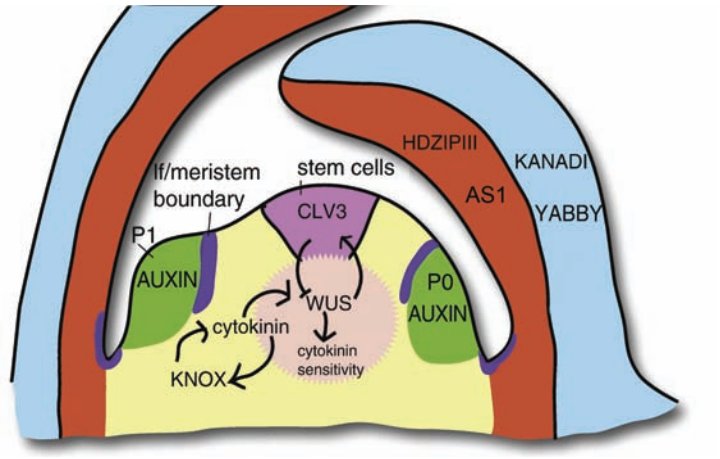
identity; A+B+E, petal (pe) identity; B+C+E, stamen (st) identity; and C+E, carpel (ca) identity. C. Schematic of one side of an Arabidopsis carpel in transverse section. The ovary walls express abaxial (light blue) and adaxial (red) identity genes. The medial meristematic ridge (mmr, yellow) is marked by KNOX expression as well was auxin peaks that also mark the eventual initiation of the ovule primordia, which are delimited by the same loci that separate primordia in the meristem (*CUC*, dark purple). The expression of other boundary genes (light purple) are involved in the differentiation of the replum (rep) and valve margins (specific features of Arabidopsis fruits). Modified from Ferrandiz *et al*., 2010 and reprinted by permission of the author. D. Schematic of a longitudinal section of an Arabidopsis ovule. The nucellus is marked by WUS expression (pink) and contains the megaspore mother cell (mmc). Both the outer (oi) and inner (ii) integuments express organ polarity genes but in distinct combinations. In the oi, abaxial identity is established by the YABBY gene *INO* along with several KANADI loci and adaxial identity involves the HDZIPIIII *REV*. In the ii, abaxial identity requires the KANADI locus *ATS* and adaxial identity appears to be patterned by multiple HDZIPIII loci (Kelley *et al.*, 2009).

Fig. 3. Schematics of different reproductive phytomers from across the seed plants. A. A simple phytomer from a male strobilus, common in many gymnosperms. The lateral organ has microsporophyll identity (open circle) and the axillary meristem is suppressed. B. A branched phytomer from the male axis of the caytonialean *Kachchia*, an example of a complex male strobilus, which is

rare among living gymnosperms. The first lateral organ is suppressed and the axillary meristem produces multiple microsporangia. C. The ovulate phytomer of a Cordaitalean. The first lateral organ is a bract. This subtends an active axillary meristem that produces several sterile scales followed by ovules (closed circles) with single integuments. The axillary meristem then terminates. D. The ovulate phytomer of *Pinus*. The first lateral organ is a bract, which represents the condensation of a shoot bearing several sterile scales, and which subtends an active axillary meristem that produces a subtending ovulate scale and two ovules each with one set of integuments (only one ovule shown). E. The ovulate phytomer of *Gnetum*. The first lateral organ is a bract whose axillary meristem produces several pairs of sterile scales or bracts, before terminating in an ovule with one pair of integuments. The most distal envelopes, subtending the ovule may in fact be the products of the ovule meristem itself. F. The ovulate phytomer of *Ginkgo*. Female short shoots produce lateral vegetative leaves with axillary meristems that give rise to a stalk with two terminal ovules, each with one set of integuments.


sepal: x4
petal: x4
stamen: x6
carpel: x2
seed: x50+
flower: x20
axillary meristem:
repeats shoot
internodes
cauline lf:x2 to 4
rosette lf: x7 to 40+
cotyledon: x2
hypocotyl: x1
made in
embryo-
genesis

A.


lf/meristem
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stem cells
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HDZIPIII
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YABBY
P1
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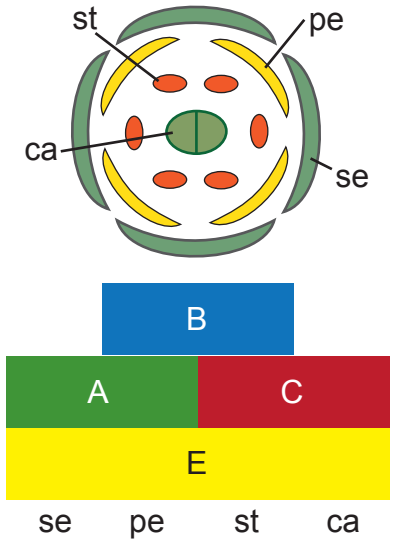
sensitivity
KNOX

B.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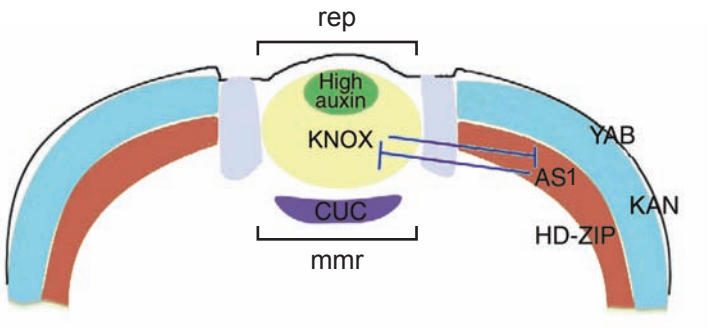
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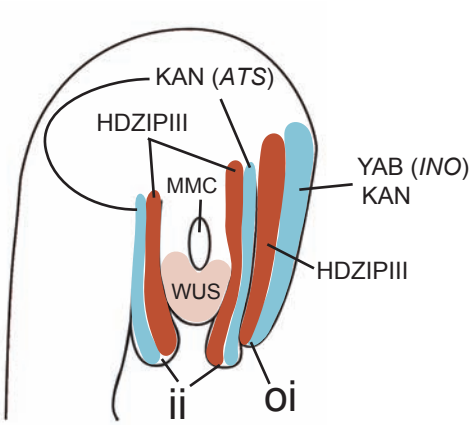
D.


KAN (ATS)
HDZIPIII
YAB (INO)
KAN
MMC
HDZIPIII
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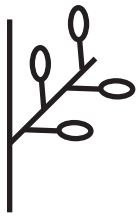
ii
oi

A. 

B. 

C. 

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E. 

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